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THE

AMERICAN NATURALIST

VOL. XXVII. October, 1893. 322

BACTERIOLOGY IN ITS GENERAL RELATIONS.[1]

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Bacteriology, although the youngest member of the biological sisterhood, has developed so rapidly within the past two decades, that to-day it may fairly claim for itself an independent basis. Independent, however, not in sense of isolation from other branches of scientific thought, but as occupying a field that is more or less distinct in itself. The scope of this subject has already widened to such an extent, and the literature grown to be so voluminous, that it is fairly entitled to be ranked as a separate branch of biology. This necessity is also emphasized by the difference in technique, that separates it more or less distinctly from other departments of research.

It was not until the middle of the present century that the affinities of the bacteria were even approximately determined. Zoologists first claimed this class of organisms as belonging to the animal kingdom, but gradually, as bacterial forms became better known, and more fully studied, their plant-like similarities were detemined. Botanists, for many years contributed but little toward a more perfect knowledge of this class, and it is the science of medicine that bacteriology must ever regard as its foster parent. The establishment of the causal relation between these minute forms of organic life and disease, at once invested them with increased importance and made them subjects of paramount interest to the student of medicine. Although the main interest that bacteriology calls forth is still in its relation to medicine, broadly considered, it has proved itself so valuable an adjunct in widely different fields, that as a science, it can no longer be subordinated to any particular subject. To-day, it may fairly be said to exert a powerful influence on a number of widely separated departments of a scientific as well as of applied nature and investigations from a bacteriological standpoint have thrown much light upon many obscure problems in these different branches.

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[1] Delivered before the Biological Club of the University of Chicago, Feb'y, 1893.

The purpose of this paper is to indicate in a general way the extent of the influence that this science is exerting on other lines of work as measured by some of the results that have been recently obtained. This can be done only in barest outline, if we would compass the entire range of its activities within the limits of a single paper. A rapid review, will however, give us a fuller comprehension of the relation that the subject as a whole bears to other departments of thought. The influence of scientific study is of a reciprocal nature, and the impetus that one branch receives from the discovery of a general principle, manifests itself to a greater or lesser degree in all related lines of study. The relation which exists between this subject in general, and medicine and hygiene must always be most intimate, for it is along these lines that the greatest advance in bacteriology has been made. It is therefore meet that in the consideration of this subject, we should first emphasize the effect that it has produced in the field of medicine, but as this is the phase that is usually presented, allusion to it here will be made only in very general terms.

Perhaps it is not too much to say, that no theory in pathology has been more fruitful of practical results than the germ theory of disease. Not only has it established the etiology of contagious maladies upon a rational and scientific basis, but it has formulated well defined principles of treatment that are now successfully employed, particularly in the departments of surgery and hygiene. What was once blindly accepted as a "visitation of Providence," from which there was no escape, is now known to be due to the action of these minute forms of organic life. To-day we consider them in the light of science rather than the gloom of superstition, and the terror of devastating pestilence is largely diminished when its cause is unmasked.

Perhaps on the whole the greatest benefit that bacteriology has conferred upon the "healing art" lies in the fulfilment of the old adage "that an ounce of prevention is worth a pound of cure." It has shown its value mainly in *preventing* rather than in *curing* disease, so that prophylaxis has gained far more than therapeutics. A more intimate knowledge of the conditions aiding or retarding the development of bacteria has resulted in improved methods of sanitation, by which the physician is able to circumscribe and control an outbreak of contagious disease.

Our knowledge of all the conditions of bacterial development is as yet comparatively limited, but the application of the principles that have already been established, has aided materially in checking the progress of these types of disease. Among these, the cholera epidemic of last year is a notable example. Starting from its native home on the Ganges, where it is endemic, it travelled step by step, following the lines of commerce. Entering Europe by way of Russia, where amid its squalid and famished millions, existed the most favorable conditions for its propagation, it was further spread by exiled Jews, who carried the contagion to many of the commercial marts of western Europe. The history of past epidemics demonstrates that its march westward has been almost always unobstructed, and had we been living in the light of two decades ago, it would probably have ravaged the denser portions of the west, as it has done in the past, and as it does in the east to-day. We may not be able to stamp out the scourge at once, but it cannot be denied that the restrictive methods of the German Government last season, checked the spread of the epidemic in that country, and our efficient quarantine regulations prevented its gaining a foothold in our metropolis. We may not know all there is to be known concerning the character of the contagion, but what we have already learned, points to a more successful method of action in repressing it than any we have heretofore possessed.

Prophylaxis, or preventive treatment, may be carried on in two ways. We may prevent disease, either by destroying the cause of the infection, or by rendering our bodies refractory to the attack of the disease germ. The first is accomplished by disinfection, the latter by preventive inoculation. What Jenner did with small-pox is an entirely empirical manner, modern bacteriology has effected in the light of established principle. The brilliant researches of Pasteur and his pupils along these lines, not only in the case of human infection as in hydrophobia, the mortality of which at the Pasteur Institute at Paris has now fallen to less than one-half of one per cent., but also certain animal diseases like splenic fever in sheep and cattle, erysipelas in swine and cholera in fowls, are practical testimonials of the advance that modern bacteriology has to offer in the direction of prophylactic treatment. The Berlin school, while it has not as yet succeeded in applying its methods in so widespread and practical a way, has placed the question of acquired immunity upon a firmer scientific basis. The discoveries of Fränkel, Brieger, Kitasato, Behring, and Ehrlich are all of supreme importance from an experimental standpoint. These observers have been able to immunize various animals by artificial means against such diseases as tetanus, diphtheria, pneumonia and erysipelas of swine. Not only have they endowed the animal body with such properties that it can successfully antagonize either the germ or its toxic product, but in some cases, have actually restored the animal to its normal healthy condition after the disease had been established.

How far these results can be utilized in combating disease in the human system is not yet definitely known. In the consideration of the probable value of these results to therapeutics, we need to be conservative in our conclusions, for the conditions are widely different between an experimental disease in animals and its natural course in man. From the present outlook, it seems that any permanent advance along this line will be based upon successful efforts in securing artificial immunity. The conditions must first be worked out experimentally upon the lower animals, and it will require patient and extended research before they can be applied to human ills. The most pronounced results so far in this connection, are accomplished by the *blood serum therapeutic methods*, as demonstrated by Behring and Emmerich. This theory rests upon the conception that in an animal artificially immuned, a certain change has taken place in the blood serum by which it has become endowed with antibacterial, as well as with antitoxic properties; also that the serum of an animal thus protected possesses in itself a positive therapeutic value. Kitasato, two years ago, succeeded in rendering mice so refractory toward the poison of the tetanus bacillus, that they were able to withstand an injection of three hundred times the ordinary fatal dose. The serum of these immuned animals when injected into susceptible controls, that had been infected with virulent tetanus cultures was able to arrest the progress of the disease even after the characteristic symptoms of tetanus had been manifested throughout the body. Not only has this course been applied to experimental tetanus in mice, but rabbits, dogs, horses and sheep, all of which are susceptible to the disease, have been rendered refractory by these artifical methods.

This plan of treatment has recently been used in tetanus in man. The curative substance was secured from the serum of artificially immuned rabbits and dogs, and there are already on record over a dozen cases of human tetanus that have been successfully treated by this method of inoculation. Some of the cases were so far advanced that paralysis had already set in, yet the injected material was able to neutralize the toxicity of the poison, and inhibit its further production. The application of this method to other diseases analogous in character promises favorable results, and the problem to-day in this connection, lies not so much in further proof of the theory, as it does in the ability to artificially immunize animals of adequate size to procure the serum in sufficient quantities to be utilized in actual practice. Behring has succeeded with diphtheria in conferring immunity upon rabbits and guinea pigs and has found that their serum is likewise able to check the experimental disease in infected controls. Subsequently he accomplished the same result in animals of the size of sheep, rendering them resistent to the infection, so that the serum in adequate quantities can be secured for use in the treatment of the human disease.

Medical science awaits with intense interest the next step, and it is possible that in the near future "the red demon of the nursery" will be brought under control.

Turning from the beneficent results which have been secured to medicine, to other lines of activity, the results of bacteriological research are equally efficient. Chief among these are some results obtained in reference to agriculture. In its relation to medicine, bacteriology deals mainly with those forms that have adapted themselves to a wholly, or semi-parasitic mode of existence. These however represent but a minor fraction of the whole number of species that have been isolated. In agriculture, saprophytic, as well as parasitic species enter into consideration, and here parasitism is not confined to the animal body as a host, but is to be observed in plant tissue as well. To the agriculturist then, bacteria come in a dual guise. They are both a friend and a foe. Some species are to him a direct benefactor, some render him aid in a more indirect manner, while others are a positive detriment to the success of his calling. How to increase the activity of the friendly forms, and diminish the ravages of the noxious ones, is the constant study of the agricultural bacteriologist.

The field, which for convenience we here classify as agricultural bacteriology, is made up of so many widely separated subjects, that the detailed methods in one class, cannot be successfully utilized in the examination of another. For example, the attempt to isolate the organism of nitrification which baffled investigators for so long a time, was fruitless mainly because they relied almost entirely upon the gelatine method of Koch. Each special subject must be studied by itself and the technique developed for the method of work in establishing the etiology of a bacterial plant, disease differs widely from that which must be used in studying the processes of nitrification or the fixation of the free nitrogen of the atmosphere. In reviewing the progress of agricultural bacteriology, we can only allude to the lines of work which have been opened up in this fertile field of research. It may be confidently asserted that as yet, we stand on the threshold of discovery in this department, and the work can scarcely be said to be more than inaugurated. What the possibilities of the future are, no man can safely predict.

Study in veterinary pathology has gone hand in hand with that of human disease, and the etiology of numerous contagious diseases in domestic animals have been thoroughly investigated. Actinomycosis in cattle, glanders in horses, and splenic fever in sheep besides numerous other septicemic diseases, like hog-cholera, swine plague, chicken cholera, *rouget* or swine erysipelas are classic examples in our textbooks. A fuller knowledge of the history and etiology of this class of diseases, has not only better enabled the farmer to check their spread, both by remedial and prophylactic treatment, but it has awakened an economic interest that is steadily progressing along practical lines.

Besides these parasites, confined to animal organisms, the husbandman has to contend with a series of forms, which only find their normal hosts in vegetable tissue. The farmer and horticulturist need to be always on the alert to protect their harvests from the ravages of these enemies. Insects and fungi are an ever present menace to success, but not a small number of plant maladies are to be traced directly to a bacterial source. Such destructive diseases as the apple and pear blight, the tuberculosis of the olive, the " yellows " of the hyacinth, as well the blight of oats, sorghum, Indian corn, and other cereals are often due to an invasion of germs of a bacterial nature. The conditions by which these diseases are propagated and spread are such, that some of them can be eradicated, now that we know how the infection is transmitted. To illustrate, the pear or fire blight, which has been observed in this country for a hundred years, and which in some sections has become so destructive as to practically ruin the industry, can now be managed with comparative ease. Through the researches of Waite, it has been ascertained that the infection is transmitted by insects in their visits to the flower for nectar. The germs thus carried from an infected to a healthy tree are deposited on the nectary as the insect garners the honeyed store. A lodgment effected, the germ finds a rich nutrient medium and it multiplies profusely, penetrating finally the exposed cells of the nectary, which are destitute of a cuticle. With an entrance once established, the contagion spreads from blossom to twig, from twig to branch, until at last the whole tree structure becomes involved, and falls a prey to the disease. Since we have learned that the blackened leaves and branches contain the virus, it becomes only a matter of watchfulness and care on the part of the husbandman to check and ultimately stamp out the malady by the excision and burning of the diseased tissue.

Another problem of vast importance not only to agriculturists, but to sanitarians, is the subject of nitrification, or the conversion of nitrogen bearing substances into nitrates and nitrites. It has been an established fact, known for years, that under certain conditions, there was an increase in the nitrous and nitric salts in the soil, but just how this was brought about, was for a long time an unsolved problem. Chemists had explained the phenomenon on a purely chemical basis, and it was believed that the nitrogen of the air was oxidized, either by the action of ozone or oxygen. Pasteur as a result of his studies on fermentation was the first to suggest that the production of nitric acid in the soil might be due to organized ferments. This idea was taken up by Schloesing and Müntz fifteen years ago, and the working out of the present accepted biological theory of nitrification is largely due to the untiring labors of these eminent French investigators, as they laid the basis for the more brilliant and successful results of Winogradsky and Warington.

Interesting as is the story, time will not permit entering upon the detailed steps by which the germ theory was finally proven. For a number of years after it had been shown that organized ferments played the chief rôle, no advance was made in the isolation of the organism as the different investigators failed to produce any nitrification with the germs they had separated from the soil. Warington states, that over a hundred different species of bacteria had each been carefully tested as to their ability to nitrify ammoniacal solutions and that with every one a negative result was reached. It is less than three years ago that the efforts of bacteriologists to obtain the specific cause of the process were crowned with success. Frankland isolated the germ by dilution, and at the same time Winogradsky succeeded in separating it by another process. The unique feature of this germ was that they could not make it grow upon gelatine. This peculiar individuality explained the failure of previous investigators who had confined their methods to gelatine media. Experiments demonstrated that this germ was only able to oxidize ammonia into *nitrous* acid. Important as was this discovery, it was only one step in the process. The conversion of nitrous acid and its salts into the more stable nitrates, eluded their best efforts.

Winogradsky at last succeeded in isolating the organism from mixed cultures by the use of gelatinous silica mixed with inorganic salts.

In this way the various steps of the process were clearly demonstrated and the biological theory of nitrification was established upon a firm and scientific basis. This theory claims that the nitrification of ammonia in the soil takes place in two successive stages, the change in each case being produced by distinct and separate organisms. In the first stage ammonia is oxidized into nitrous acid and then this is acted upon by another (a second) organism and is converted into a nitrate. Ordinarily, as observed in nature, the two processes occur simultaneously so that the ammonia passes in continuous combination from its simpler form into its ultimate stage as nitric acid, or some of its salts. The economic value of this discovery cannot be estimated. It is not only a complete and satisfactory scientific solution of a complex process, but is of invaluable aid to the agriculturist in demonstrating to him the source of the most essential element in soil restoration and in teaching him how he can best conserve and utilize this expensive product.

It has also an important bearing upon certain phases of hygiene for the success of the intermittent filtration of water through the soil seems to be based upon the nitrifying action which takes place within the filter. Filtration has been in use a long time as a means of water purification and the theory that bases its efficiency in the mechanical or chemical separation from its impurities has long been the accepted one.

Many of the observed facts did not seem however to accord with this explanation, and when it was found that certain protoplasmic poisons like chloroform injuriously affected the efficiency of filters, the relation between it and living organisms was suspected. This led to a careful study of the question from a bacteriological standpoint, and the discovery was soon made that there was a direct relation between the process of nitrification as it went on in the filter and its effectual operation.

The solution of another interesting subject, that of the fixation of the free nitrogen of the air by certain plants, also bids fair to be solved by bacteriological methods. Agriculturists long ago recognized the fact that certain species of plants belonging to Leguminosæ, such as clover, alfalfa, etc., possessed special properties in enriching the soil; that not only could these crops dispense with the application of fertilizers, but that they possessed the elements by which impoverished soils might be restored to their original fertility quicker and cheaper than by any other method. This knowledge, the fruitage of observation and experience awakened an interest in the causal relations between the one and the other, productive of many interesting and ingenious hypotheses. Liebig concluded that as clover could be grown upon the same ground for years without the addition of fertilizers, that it must absorb nitrogen direct from the air through its aerial organs, and then transfer this store to the roots, and so enrich the soil. Carefully conducted experiments have failed however to reveal any direct absorption by the leaves and stems of plants. This peculiar property, limited so far as we at present know, to the Leguminosæ was finally correlated with the presence of small excrescences or tuberculous swellings on the roots of these plants. These are found in great profusion on the young rootlets of this class of plants, and seem to be universally absent in all other genera.[2]

For a long time they were regarded as pathological structures, and it was only when it was found that legumes grown in *sterilized soil* were poorly nourished and entirely devoid of tubercles, that the causal relation of these structures with the peculiar property of the plant was recognized. These observations were followed by more systematic experiments by Hellriegel and Wilfarth, who discovered that the production of the tubercle depended upon the germ contents of the soil. Beyerinck, Prasmowski and others succeeded in isolating the germ which possessed the ability to penetrate the young root hairs and the cortical parenchyma of succulent roots. These bacteria set up an irritation in the plant tissue that causes the formation of a meristem, and thus the tubercles are produced as lateral outgrowths from the root.

The tubercle is filled with peculiar shaped bacteria, which possess the ability in some way not yet perfectly understood, of fixing the nitrogen of the air in such a condition that it can be utilized by the plant. Our knowledge concerning the details of the process is far from complete, but this rational explanation of the phenomena has opened the way for further research. The problem that at present awaits solution is to find whether there is any difference between the organisms infecting one variety and those that are symbiotic in another.

The artificial selection and cultivation of these forms that have the greatest ability as "nitrogen collectors" is a question of prime importance. Like that of nitrification it involves great economic interests, inasmuch as it is the key to the solution of the important question of the restoration of impoverished soils.

It would be treating our subject in a very imperfect manner were we to close our résumé of the influence that bacteriology has exerted upon argricultural pursuits without stating the position of it with reference to dairying.

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[2] Recent experiments (Landw. Vers. Stat. XLI, 138) seem to indicate that the tubercles on the roots of *Elæagnus angustifolia*, a member of the Elæagnaceæ, a close relative to the Leguminosæ are developed by bacteria. Infection by means of soil washings of sterilized soil planted with these seedlings gave more luxuriant growth than controls in sterilized soil. The organism has been isolated in pure culture and is found to differ from *Bacillus radicicola*, the form so common in the legumes.

Milk, like every other organic substance, is subject to certain changes, and these conditions are now known to be directly traceable to the influence of micro-organisms. These so-called "diseases" of milk are mainly fermentative in their character and modern bacteriology has shown the dairyman that they can be entirely prevented if he handles his product in a rational way. Milk, as it comes from the cow, is entirely devoid of germ life, but its high degree of temperature when freshly drawn and the exceedingly nutritious food medium that is offered, afford the optimum of conditions for the development of any bacteria that find their way into the fluid. The ubiquity of distribution of fermentative organisms render it impossible to entirely avoid their action, but the losses of the milk-dealer can be greatly diminished if cleanliness is scrupulously observed.

While the milkman wishes to avoid bacteria as far as possible, the buttermaker should welcome them as his friend. Many forms are, of course, undesirable, but from the recent investigations in this field there is no question but that the bacterial content of butter largely influences its keeping qualities as well as the peculiar flavor for which such fancy prices are paid. The process of "ripening" or "souring" cream that is practiced by every butter maker is a natural bacterial fermentation. Better and more butter is obtained from ripened than from "sweet" cream. If this is so, the question naturally arises, Why can we not isolate the forms that are capable of prorucing these changes and add them directly to the cream rather than to trust to their spontaneous development? The researches of Hansen in the realm of pure yeast ferments have revolutionized the brewing industry within the last five years, and the introduction of pure cultures of yeast for fermentative purposes has made a better, and what is still more important, a more uniform product. Now the same field seems to be opening in the process of butter-making. Danish and German creameries are now being supplied with pure bacterial cultures that are early added to the cream in order to produce as a result of the fermentative changes the desired flavor.

In this way uniformity of product is assured, and as many undesirable forms are choked out by the introduction and control of pure cultures, the keeping qualities of the butter are much improved.

The utility of bacteria in connection with milk and its products may still be further extended, for the necessity of their presence in cheese making is even more imperative than in butter making. Here they are an indispensable requisite to the production of a palatable product. New cheese is flat and insipid, and it requires a certain period of time in which it undergoes a change that is called the ripening or maturing stage before it is fit for use. Duclaux and others have shown that the production of the delicate flavors are due to the transformation of the casein into soluble albuminoids by means of the ferments that are produced as a result of bacterial growth. This ripening process is then due to the action of specific germs. The various kinds of cheeses that possess such a variety of flavors are, no doubt, due to the action of different forms of bacteria, but the threshold of this work is scarcely more than crossed, and, as one of the leading experts in this country has said, further advance in perfecting the processes of cheese manufacture is impossible, unless they include the aid of bacteriology.

(*To be continued.*)